

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

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## Quick links for frequently used directions:

Verifications	Maintenance	Performance Checks
<a href="#">Buffer G2</a>	<a href="#">Qiagen Biobot EZ1</a>	<a href="#">EZ1 Pipetting Accuracy</a>
<a href="#">Buffer MTL</a>	<a href="#">QIAcube</a>	<a href="#">EZ1 Leakage</a>
<a href="#">GenTegra-DNA</a>	<a href="#">Thermo-mixers</a>	<a href="#">EZ1 Temperature Accuracy</a>
<a href="#">DTT</a>	<a href="#">7500 RT-PCR</a>	<a href="#">ProFlex Performance Check</a>
<a href="#">EZ1 DNA Investigator kits</a>	<a href="#">ProFlex thermal cyclers</a>	
<a href="#">Fast Blue B</a>	<a href="#">3500 – weekly/monthly</a>	
<a href="#">GlobalFiler kit</a>	<a href="#">3500 capillary change</a>	
<a href="#">GlobalFiler Express kit</a>	<a href="#">EZ1 log form</a>	
<a href="#">PSA ABA cards</a>	<a href="#">QIAcube log form</a>	
<a href="#">PowerPlex Y23 kit</a>	<a href="#">Thermo-mixer log form</a>	
<a href="#">Prep-n-go Buffer</a>	<a href="#">7500 log forms</a>	
<a href="#">Quantifiler Trio kit</a>	<a href="#">3500 form</a>	
<a href="#">Sterile water</a>		
<a href="#">TE buffer</a>		
<a href="#">Verification form</a>		

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**Section 1 Chemicals and Reagents****1.1 Introduction**

By definition, “critical reagents are determined by empirical studies or routine practice to require testing on established samples before use on evidentiary or casework reference samples” (FBI QAS, 2009). Reagents which are used in pre-amplification procedures directly involved in DNA extraction from forensic casework or database samples, have been deemed critical reagents to prevent unnecessary loss of sample. Except for allelic ladders, all post-amplification DNA reagents are hereby listed as non-critical reagents; allelic ladders are critical reagents.

Non-critical DNA reagents need not be verified prior to use in casework.

When a reagent fails to meet the criteria for verification, the DNA Technical Manager shall be notified, and an appropriate course of action will be determined. The reagent shall not be used in casework unless or until the issue has been resolved and the approval or an alternate course of action suggested by the DNA Technical Manager has been documented.

**1.2 General Instructions**

- Chemical and reagent quantities may be adjusted to prepare more or less than the specified amount.
- All critical reagents prepared in-house shall be stored in sterile/autoclaved containers.
- Reagent containers are to be labeled with the following:
  - Name of reagent
  - Lot number (the date of preparation and preparer’s 2 or 3 letter initials are used as the lot # for reagents prepared in-house and reagents where a lot # is not provided by the commercial vendor, i.e., 06-0101MLC would be the lot # for a reagent prepared on Jan. 1, 2006 by MLC)
  - Expiration date
  - Reagents prepared or removed from their primary container for daily use need only be labeled with the identity of the reagent and the date and initials of the scientist that prepared or is using the reagent.
- One member of the DNA discipline shall be designated for purchasing of supplies and reagents.
- All chemicals and reagents prepared or purchased shall be logged in the reagent log maintained in the DNA laboratory.
- All purchased chemicals/reagents are assigned the expiration date specified by the manufacturer. If no manufacturer expiration date is provided, the following guidelines apply:
  - Chemicals used in the in-house preparation of a reagent are not assigned an expiration date. Expiration dates are assigned to the prepared reagents as specified below.
  - Reagents used as received will expire one year from the date of receipt.

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- All newly received/prepared critical reagents and chemicals shall be verified prior to use on casework/database samples. Chemicals/reagents requiring verification should be clearly marked as such.

**1.2.1 Special handling considerations for STR and Y-STR PCR kits**

As per vendor communications, it is possible that the outer packaging of PCR kits could potentially be contaminated with allelic ladder. To minimize the risk of introducing allelic ladder contamination into pre-PCR workspaces, additional precautions must be used when receiving and un-packaging PCR kit components.

- Disassembly of the PCR kit must take place in the vestibule outside the PCR room.
- Disposable gloves must be worn and changed frequently during the kit disassembly process.
- Avoid placing the kit in direct contact with table surfaces – use a disposable barrier (i.e. bench paper or bench towels) to cover the bench top.
- The outer layer of clear plastic wrapping should be treated as if it could be contaminated with allelic ladder. Remove the plastic wrapping with the following procedure:
  - Use single-use scalpel and forceps to open and peel back the plastic
  - Discard scalpel and forceps
  - Change gloves
  - Remove the inside Pre-PCR box(es) to a separate covered bench area , making every attempt to avoid contact with the outer surface of the plastic wrapping
  - Discard plastic wrap
  - Change gloves

Pre-PCR reagents (Master Mix, Primer Mix, 007 Control DNA):

- Set up a tube storage box on a clean bench paper
- If the pre-PCR reagents are in an additional layer of plastic wrap, follow the above procedure for removing the next layer of plastic wrap.
- Open the box of pre-PCR reagents
- Change gloves
- Minimizing contact with packaging, remove reagents into the tube storage box.
- Change gloves
- Close the tube storage box, label, and move it to its correct storage location
- Discard packaging materials and bench paper; use bleach to clean the work surface; change gloves

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## Post-PCR reagents (Allelic ladder):

- Set up areas inside the PCR room for further handling, including one bench paper for removal of plastic wrap and an adjacent area for receiving the inner contents
- Remove gloves before returning to vestibule; put on clean gloves in vestibule
- Bring the post-PCR reagents into the PCR room.
- Change gloves
- Use the plastic wrapping removal procedure described above
- Open the box containing tubes of allelic ladder
- Change gloves
- Minimizing contact with packaging, remove tubes of allelic ladder into a tube storage box.
- Change gloves
- Close the tube storage box, label, and move it to its correct storage location
- Discard packaging materials and bench paper; use bleach to clean work surface; change gloves

**1.3 Chemicals and Reagents not Requiring In-House Preparation and/or Verification**

Chemicals/Reagents purchased from a commercial vendor and requiring no preparation or verification prior to use in procedures or preparation of other reagents are listed below. They shall be stored as prescribed by the manufacturer and shall expire on the date provided by the manufacturer. Expiration dates are assigned as previously described, if not provided by the manufacturer and unless stated otherwise.

- 7500 RT PCR RNase P plate [liquid]
- $\alpha$ -Naphthyl Phosphate [solid]
- Aluminum Sulfate [solid]
- Anode Buffer Container, 3500 series from Life Technologies [liquid]
- Bleach [liquid]
- Cathode Buffer Container, 3500 series from Life Technologies [liquid]
- Conditioning Reagent, 3500 series from Life Technologies [liquid]
- Dithiothreitol [solid]
- Electrode Filling Solution [liquid]
- Electrode Storage Solution [liquid]
- Ethanol, anhydrous reagent grade [liquid]
- Fast Blue B (o-Dianisidine Tetrazotized) [solid]
- GeneScan 600 Liz Size Standard [liquid]
- Glacial Acetic Acid [liquid]
- Concentrated Hydrochloric Acid (HCl) [liquid]
- Hydrogen Peroxide [liquid]
- Identifiler Allelic ladder

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- Indigo Carmine dye [solid]
- Multi-Capillary DS-36 Matrix Standards (Dye Set J6) [liquid]
- Multi-Capillary DS-33 Matrix Standards (Dye Set G5) [liquid]
- Nuclear Fast Red [solid]
- pH 4, pH 7, and pH 10 buffers [liquid]
- Phenolphthalein [solid]
- Phenolphthalein solution [liquid]
- POP-4 Polymer from Life Technologies [liquid]
- Promega Matrix 5C Standard
- Potassium Hydroxide [solid]
- Saturated Picric Acid [liquid]
- Semen Standard [liquid]
- Sodium Acetate, anhydrous [solid]
- Sodium acetate buffer solution (3M, pH 5.2) [liquid]
- Sodium Hydroxide Solution (NaOH) [liquid]
- Sterikon<sup>®</sup> plus Bioindicator [ampules]
- Tris base [solid]
- Xmas Tree Stain [liquid]
- Xylene Substitute [liquid]
- Zinc [solid]

#### 1.4 Preparation and Verification

- Unsupervised verification can only be performed by trained, competency tested and authorized scientists.
- Vendor supplied standard samples / positive control samples that are sent with PCR amplification kits may be discontinued or substituted at vendors' discretion. The batch central log information will indicate the identity of positive control samples used in analysis.
- Similarly, variations in vendor supplied materials (changes instituted by the vendor and outside of laboratory control) will be assessed to determine if the change adversely affects the laboratory analysis in which the reagent/chemical is used. This assessment will also be documented in the verification paperwork. Kit component information in the chemicals and reagents section will be updated as required when the manual is revised.
- Verification of a reagent that is only used as a component of another reagent is achieved by verifying the final preparation and does not need to be documented separately.
- Reagents used in the same procedure may be verified simultaneously. If the verification fails, the components will then need to be verified separately.
- Verification paperwork is maintained by calendar year in SharePoint and shall include the DNA Critical Reagent Verification Form, for critical DNA reagents.
- For successful verification of screening reagents, the positive and negative controls must perform as described in the Forensic Biology Casework

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Procedures Manual. Reagents must be successfully verified prior to use in casework.

- Verification must be performed using the most stringent conditions routinely encountered in casework, including GenTegra dry-down and re-hydration in minimum amplification volume where applicable.
- For verifications that include amplification and electrophoresis, the paperwork consists of the electropherograms for the positive control/reference sample(s) and negative control/blank(s). Verification results are assessed as described in the Interpretation section of the Forensic Biology Casework Procedures Manual. The expected results must be obtained for a chemical/reagent to be successfully verified and appropriate for use in casework/database analysis.
- For verifications that include peak height assessments, a copy of the peak height assessment must be included in the verification documentation.
- In the verification of casework amplification kits, the relative fluorescence units (RFU) for the known sample amplified with the new kit are compared to the results obtained with the kit currently in use to estimate the sensitivity of the new kit. This is important for adjusting the target value with the new lot of kits.
- The central log paperwork for verifications may be referenced by noting the batch in which the verification was performed.
- Upon successful verification, the reagent log shall be updated with the verification date and scientist, and the storage location for the reagent.
- When verification fails on a reagent prepared in-house, the reagent may be re-prepared and/or verification repeated. If verification fails again, consult with the DNA Technical Manager to determine the appropriate course of action. For purchased reagents/chemicals, the DNA Technical Manager shall be consulted to determine the appropriate course of action.

## Preparation and Verification of Reagents and Chemicals

### Buffer G2

**(DNA critical reagent)**

(when purchased outside of a kit)

Purchased from Qiagen and stored at room temperature

#### Verification

Extract, GenTegra, amplify, and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of buffer.

### Buffer MTL

**(DNA critical reagent)**

Purchased from Qiagen and stored at room temperature

#### Verification

Extract, GenTegra, amplify, and analyze a previously typed reference sample and a corresponding reagent blank using the new lot of buffer.

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**GenTegra-DNA****(DNA critical reagent)**

Purchased from GenTegra and stored at room temperature prior to hydration, stored at 2-8°C following hydration. Expiration date of the dried form is manufacturer's expiration date. Expiration date of the hydrated GenTegra-DNA stock is three months after prep date.

Verification

The verification process is described and documented on the GenTegra-DNA Verification Form.

**DTT (1M)****(DNA critical reagent)**Working Solution

Dissolve 0.77g dithiothreitol in 5mL sterile de-ionized water in a sterile conical tube. Add 50µL of 3M Sodium Acetate buffer solution, pH 5.2. Do not autoclave. Aliquot (0.5 – 1.0 mL recommended) and store at -15 to -25°C. Aliquots expire one year from date of first thaw.

Verification

Extract, GenTegra, amplify, and analyze a previously typed semen sample and a corresponding reagent blank using the new DTT lot. Verification may be performed using either a differential or a direct with DTT extraction procedure.

**EZ1 DNA Investigator Kit****(DNA critical reagent)**

Components: Reagent Cartridges, Buffer G2, Proteinase K solution, carrier RNA

Purchased from Qiagen and stored at room temperature.

Carrier RNA solution is prepared by reconstituting the carrier RNA in 310µL of sterile, de-ionized water. Vortex and spin briefly. Prepare 20µl, single use aliquots in 0.5mL tubes and store at -15 to -25°C. Reconstituted carrier RNA expires one year from date of preparation.

Verification

Extract, GenTegra, amplify, and analyze a previously typed reference sample and a corresponding reagent blank using all components from the new kit lot.

**Brentamine / Fast Blue B**Solution #1

Dissolve 10 mg α-Naphthyl Phosphate in 10 mL deionized water.

Solution #2

Dissolve 2.5 mg Fast Blue B (o-Dianisidine Tetrazotized) in 10 mL sodium acetate buffer (0.14 M, pH ~5.0).

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Store both solutions at 2-15°C; solution expires 7 days from date of preparation.

Alternatively, these reagents may be made in bulk, aliquotted and frozen. Frozen reagents expire one year from date of preparation; thawed aliquots expire one day from date of thaw.

Verification

Test the reagent with a positive semen control and a negative dH<sub>2</sub>O control prior to first use, and on each day used in casework.

**GlobalFiler Amplification and Typing Kit (DNA critical reagent)**

Components: GlobalFiler Master Mix, GlobalFiler Primer Set, GlobalFiler Allelic Ladder, DNA Control 007

Purchased from Applied Biosystems. Stored at -15 to -25°C upon receipt and until needed, stored at 2 to 8°C after initial thaw for up to 6 months or up to the expiration date stated on the kit (whichever comes first).

Verification

- The verification procedure is detailed and documented on the GlobalFiler Verification Form
- Results must be submitted to the Technical Manager for approval of the kit. Average peak height must be within validation range specific on verification sheet, and peak height variation between old and new kits should be within 30%. The DNA Technical Manager will monitor performance among GlobalFiler kit lots.
- The course of action for a kit that fails verification will be determined by the Technical Manager.

**GlobalFiler Express Kit (DNA critical reagent)**

Purchased from Life Technologies and stored at -15 to -25°C until thawed, then stored at 2-8 °C. Expiration date is either six months from date of thaw or manufacturer's expiration date, whichever comes first.

Components: DNA Control 007, Master Mix, Master Mix Additive, Primer Set and GlobalFiler Express Allelic Ladder.

Verification Procedure

Amplify DNA Control 007 and a corresponding negative amplification control using the master mix, master mix additive, and primer set; and analyze using GlobalFiler Express Allelic Ladder.

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**Hi-Di Formamide**

Purchased from Life Technologies. Aliquot (0.5mL and 1.0mL recommended) and store at -15 to -25°C. Aliquots are intended for one-time use and should not be re-frozen.

**Nuclear Fast Red stain**

Note: Alternatively, this reagent may be purchased.

Dissolve 5.0g of aluminum sulfate in 100ml of hot deionized water (~40°C). Add 0.1g of Nuclear Fast Red. Stir and let cool. Filter the solution and store at room temperature; expires one year from date of preparation.

**One-step PSA ABACards**

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

A known human semen standard and sample blank are to be run to verify a new lot(s) of cards. Pooled human semen is spotted onto a stain card as a mock semen stain. The sample is processed like a casework sample, as described in the Forensic Biology Casework Procedures manual (FBPM, current version). Record the lot number(s) and expiration date(s) and test results.

**One-step HemaTrace ABACards**

Purchased from Abacus Diagnostics. Stored according to manufacturer's instructions.

Verification

A known human blood standard (positive control) and a negative control (extraction buffer or deionized water) are run to verify a new lot(s) of cards. Follow the test procedure described in the Forensic Biology Casework Procedures. Record the lot number(s) and expiration date(s) and test results.

**Permunt**

Purchased from a commercial vendor and stored at room temperature.

Working Solution: Permunt diluted with Xylene substitute if necessary. Use until no longer functioning adequately as a mounting medium.

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**Phenolphthalein (for Kastle-Meyer Test)**

Note: Alternatively, this reagent may be purchased.

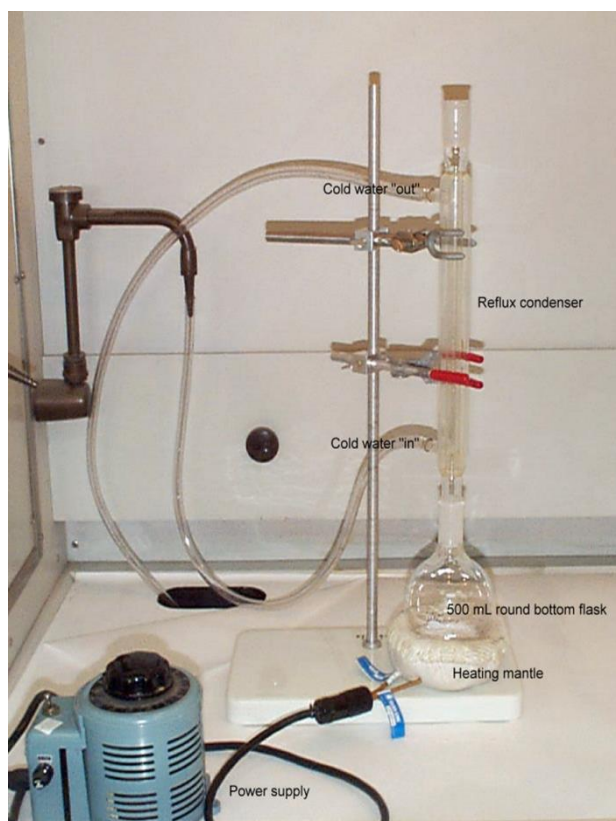
Stock Solution

Reflux 2g phenolphthalein, 20g potassium hydroxide, and 100mL deionized water with 20g of zinc until the solution becomes colorless (approximately 30 minutes to 1 hour after boiling begins – See Figure 1). Store the solution at 2-8°C in a dark bottle to which some zinc has been added to keep it in the reduced form.

Working Solution

Combine 20mL phenolphthalein stock solution (obtained from the biological screening discipline) with 80mL Ethanol (anhydrous reagent grade). The solution is stored at 2-8°C in a dark bottle. This reagent has no expiration date and may be used as long as the appropriate reactions are observed with the positive and negative blood controls, prior to use on evidentiary items.

**Figure 1. Phenolphthalein Stock Solution Preparation.**



- Assemble the reflux apparatus as shown.
- Turn on cold water at source. Allow the system to fill and cool. Adjust flow so that no bubbles are formed in the condenser.
- Add the chemicals, deionized water and zinc to the 500mL round bottom flask.
- Reassemble the apparatus. Place the flask on the heating mantle.
- Turn on the power supply. Heat the flask to a gentle boil (100°C for approximately 15 minutes)
- Adjust temperature setting to 75°C and allow the solution to reflux until colorless (approximately 2-3 hours).
- Store the solution with the zinc from the flask at 2-8°C in a dark bottle.
- Clean glassware with EDTA and water.

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**Picro-indigo-carmin stain**

Note: Alternatively, this reagent may be purchased.

Add 0.33g of Indigo Carmine dye to 100mL of saturated picric acid. Store at room temperature; expires one year from date of preparation.

**PowerPlex Y23 Kit****(DNA critical reagent)**

Purchased from Promega and all except 2800 control DNA are stored at -15 to -25°C until thawed, then stored at 2-8 °C. 2800 control DNA is always stored at 2-8 °C. Expiration date is either one year from date of thaw or manufacturer's expiration date, whichever comes first.

Components: DNA Control 2800, Master Mix, Primer Set, WEN ILS 500 Y23, and PowerPlex Y23 Allelic Ladder.

Verification

- The verification procedure is detailed and documented on the PowerPlex Y23 Verification Form
- Results must be submitted to the Technical Manager for approval of the kit. Average peak height must be within validation range specific on verification sheet, and peak height variation between old and new kits should be within 30%.
- The course of action for a kit that fails verification will be determined by the Technical Manager.

**Prep-N-Go Buffer****(DNA critical reagent)**

Purchased from Life Technologies and stored at room temperature.

Verification

Amplify and analyze a previously typed reference sample and a corresponding positive and negative amplification control using the new lot of buffer.

**Proteinase K Solution****(DNA critical reagent)**

(when purchased outside of a kit)

Purchased from Qiagen or another suitable vendor and stored at room temperature

Verification

Extract, GenTegra, amplify, and analyze a previously typed reference sample and a corresponding reagent blank with the new Proteinase K lot.

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**Quantifiler Trio Kit****(DNA critical reagent)**Components: PCR Reaction Mix, Primer, DNA Standard, Dilution Buffer

Purchased from Life Technologies. All reagents received and stored at –15 to -25 °C until thawed for first use. Once thawed, reagents are stored at 2-8 °C. Standard curves have an expiration date of two weeks after date of preparation.

Verification

- The verification procedure is detailed on the Quantifiler Trio Verification Form.
- Acceptable criteria are defined in FBPM. Follow the procedure defined in that section for non-passing results.
- Document the passing human and male Y-intercept values in the 7500 instrument logbook.
- Submit the Quantifiler Trio Verification Form and the Experimental Results Report to the DNA Technical Manager for approval.

**Sodium Acetate Buffer (0.14 M, pH ~5.0)**

(for FBB preparation)

Dissolve 1.2 g Sodium Acetate (anhydrous) in 100 mL deionized water. Adjust the pH to 5.0 with glacial acetic acid. Store solution at room temperature; expires one year from date of preparation.

**Sterile De-ionized Water (H<sub>2</sub>O)****(DNA critical reagent)**

Fill glass bottles with nanopure de-ionized H<sub>2</sub>O. Autoclave (alongside a Sterikon™ plus Bio-indicator, or equivalent) for 30 minutes and store at room temperature. Expires 1 year from date prepared.

The autoclaved ampoule and a control ampoule that are placed in an incubator (at ~56°C) for a minimum of 48 hours. Evaluate as per manufacturer's instructions. Seek Technical Manager guidance when the autoclaved ampoule does not perform as expected.

The DNA Technical Manager must approve use of reagents autoclaved without a Sterikon™ (or equivalent). This approval will be documented in the Reagent Log.

Verification

Extract a previously typed reference sample and corresponding reagent blank using the direct extraction for known samples procedure. GenTegra and amplify both extracts.

**TE<sup>-4</sup> Buffer****(DNA critical reagent)**

Purchased from Life Technologies, aliquotted, and stored at room temperature.

Verification

Amplify and analyze a previously typed reference sample and a corresponding positive and negative amplification control using the new lot of buffer.

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**Section 2 General Laboratory Maintenance**

The Forensic Biology Staff are responsible for the housekeeping in the laboratory and for the routine maintenance of equipment and instruments. These tasks are delegated to a designated scientist. Other discipline members will assist as needed. Log sheets for maintenance and housekeeping are completed as appropriate.

- Receipt of packages and logging of chemicals/reagents.
  - Indicate date received on packing slip, initial and provide to the unit supervisor.
  - Unpack contents, label with date received and initials, store them in the proper location, record in logbook
  - Label with “needs verification” stickers and note on board that verification is required (if appropriate)
- Clean laboratory common spaces as needed (post-PCR lab cleaned every three months at a minimum, all other labs monthly at a minimum), wiping down counters, computers, centrifuges, phones, door handles, etc. with 10% bleach. Each scientist is responsible for bleaching his/her own personal computer and workspace after use and as needed.
- UV PCR set-up hoods for 30 minutes after use.
- Wipe down equipment/instruments as used
- Reboot genetic analyzer computers weekly.
- Sweep and mop floors as needed (every three months at a minimum)
- Bleach wipe down of communal safety hoods
- Perform weekly, monthly, and semi-annual maintenance on instruments.
- Defragment instrument computer hard drives monthly.
- Put away clean laboratory dishes as needed.
- Keep both labs well stocked and inform the designated discipline purchasing agent of reagents and supplies that need to be ordered.
- Replenish reagents on genetic analyzers, as needed.
- Autoclave water as needed. Make sure that new kits/reagents are verified in a timely fashion.
- Autoclave consumables (tubes, toothpicks, etc.)

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**Section 3 Equipment / Instrument Maintenance**

All maintenance and performance check records are maintained with the instrument, unless otherwise specified. Each calendar year, records are archived in the annual Forensic Biology case record in SharePoint.

When laboratory equipment is placed out of service for any reason, a note will be made in the equipment/instrument maintenance log (if applicable, as not all equipment has a maintenance log) and the equipment clearly marked with a note to alert scientists not to use the equipment until further notice. Unless otherwise noted, routine maintenance and performance checks are not required while an instrument is out of service.

Equipment/instrument manuals referenced in this section are either available online, on the laboratory network or in the designated location in the Forensic Biology discipline. Instrument manuals will be retained indefinitely. Equipment manuals are not required and do not need to be retained for equipment no longer in use.

Critical equipment is noted in the sub-headings below. New items of critical equipment require a performance check before use in casework analysis.

**3.1 Temperature Logs**

Temperatures for refrigerators/freezer that contain chemicals, reagents and evidence are monitored electronically as a component of the laboratory security system. Temperatures for incubators are recorded by the scientists, when equipment is in use.

The discipline supervisor or DNA Technical Manager will be notified (by the lab manager or maintenance specialist) if a temperature falls outside of the acceptable range. Temperatures may be out of range following a prolonged period of the unit's door being opened. If the temperature falls outside of the acceptable range and is not corrected by a later second reading or a minor adjustment of the unit's temperature control, the DNA Technical Manager is consulted to determine a course of action.

**3.2 Microscopes**

Reference: <http://www.leica-microsystems.com/>  
*Leica DM1000/Leica DM1000 LED Operating Manual*

**General Instructions**

- Simple dust is the number one enemy of microscopes and optical quality. When the scope is not in use, it should be covered with a plastic dust cover. Never leave a tube or an objective port open so that dust can get to the internal surfaces.

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- When cleaning of the microscope stand is required, use a clean, lint free cloth lightly moistened with water containing a small amount of mild detergent. Quickly follow the cleaning by wiping with a dry lint free cloth.
- Any residue of mounting medium or immersion oil on the stand or stage should be removed immediately after examinations are completed, using a cotton-tipped swab or cloth lightly moistened with xylene substitute. Following this solvent cleaning, the xylene substitute should be removed as quickly as possible using a clean, dry cloth. It is wise to follow the solvent removal with the above detergent cleaning.
- Before any physical contact is made with the lens surfaces (eyepieces, objectives, condenser, field diaphragm), any loose dust or debris should be blown off using compressed gas. Any stubborn dust, dirt or oil can be removed using lens cleaning fluid and a cotton-tipped swab.
- Proceed to clean the lens with a moistened swab by placing the tip at the center of the lens and working with light pressure toward the outside of the lens in a spiral motion. Immediately repeat this process using a dry swab. For very small objective lenses, the swab may be gently rotated between the thumb and forefinger while it contacts the lens. Examine the surface of the lens in reflected light for any evidence of smearing; if the surface is not completely clean repeat the process. When clean, a coated lens will have a uniform bluish color. It may be necessary to use a small amount of xylene substitute to remove oil or other mounting mediums (see above).
- Scopes should be cleaned, lubricated and aligned when necessary by a competent microscope mechanic.
- If artifacts caused by dirt are seen in the microscope image, one can locate their source in the following manner:
  - If the trouble can be eliminated by a slight adjustment of the condenser, look for the cause in the lamp bulb, lamp condenser, or filter in front of it.
  - If a change of focus control eliminates the artifact, look to the condenser or specimen itself.
  - If rotation of the objective lens causes the artifact to move, the soil is obviously on the objective. Similarly, if rotation of the eyepieces causes the artifact to move, the soiling is on the eyepiece.

Operation / Troubleshooting / Maintenance

See referenced manuals.

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**3.3 Thermo Scientific Orion Star A111 Benchtop pH Meter and Electrode**

*Reference: Thermo Scientific Orion Star A111 Benchtop pH Meter Reference Guide  
Thermo Scientific Refillable Ag/AgCl pH Electrode User Guide*

Operating Instructions

- Prior to use in reagent preparation, prepare and calibrate the electrode as described in the referenced User Manual.
- The calibration buffer should be selected to be near the pH of the reagent being prepared.
- Calibration is recorded on the log provided at the back of this manual and is maintained with the equipment.
- Calibration records are archived annually in SharePoint.

Maintenance and Troubleshooting

- See referenced manual.

**3.4 Mettler Toledo XS204 Analytical Balance**

*Reference: Excellence Analytical Balances XS Models Operating Instructions*

Analytical balances are calibrated annually. Records of calibration and maintenance are archived annually in SharePoint. The weight set is calibrated every year.

Semi-annual performance checks are performed approximately every 6 months, prior to the EZ1 Advanced XL performance checks. The check is recorded on the form provided at the back of this manual.

**3.5 Qiagen BioRobot EZ1 Advanced-XL****(critical instrument)**

*Reference: EZ1 Advanced XL User Manual*

*Qiagen supplementary protocol MA67 (Evaluating pipetting accuracy of the EZ1® Advanced XL using the EZ1 Advanced XL Test Card)*

*Qiagen supplementary protocol MA68 (Evaluating the temperature accuracy of the EZ1® Advanced XL)*

Maintenance Procedures

Preventive Maintenance procedures are described in Section 6 of the EZ1 Advanced XL User Manual and recorded on the log provided at the back of this manual. Regular maintenance is performed after each run and includes cleaning the piercing unit and wiping down work surfaces in the instrument (reference 6.1 in User Manual). Daily maintenance is performed at the end of each day the robot is in use and includes inspecting the O-rings to ensure they are clean and intact, 30 minute UV decontamination as described in Section 5.7 of the User Manual, and wiping down the instrument (reference 6.2 in User Manual). Note: It is only necessary to clean other interior surfaces in the EZ1 with dilute neutral soap solution in instances where a spill or splashing has occurred.

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**NOTE:** EZ1 Advanced XL instruments have a UV lamp life of 1500 cycles. The instrument will give a warning when the lamp needs to be replaced (50 cycles remaining). Notify the discipline supervisor if this warning is received.

O-rings will be greased (refer to section 6.3 of the User Manual) during the last week of the month (+/- one week).

Any preventive maintenance (PM) and service to the instrument, as well as the dates that an instrument is taken out of service or returned to service are also recorded.

**Performance Checks**

Performance checks shall be run bi-annually, regardless of whether or not service was performed on the instrument. Additionally, any instrument having PM or service performed shall be subjected to a performance check prior to being used again for casework analyses.

Procedure: Performance checks include pipetting accuracy, leakage test, and temperature accuracy. These are performed in accordance with Qiagen supplementary protocols MA67 and MA68 and are documented on the Maintenance Log.

Evaluating results: Acceptable ranges for each test are also listed on the performance check forms.

Pipetting accuracy - 100µL of water (acceptable range 92-108µL)

Pipetting accuracy - 500µL of water (acceptable range 460-540µL)

Leakage test – no dripping

Temperature accuracy test - measured temperature is within +/- 3°C of 60 °C

Unacceptable data: If a test fails, repeat the test. If it fails a second time, mark the instrument as offline on the instrument itself and in its logbook, and notify the DNA Technical Leader to determine a course of action.

Documentation of completion and approval/rejection: The forms for evaluating and recording the results of a performance check (four pages) are located at the back of this document, or as freestanding spreadsheets (which automatically perform calculations). Printouts of completed forms, including a pass/fail assessment, are kept in the EZ1 maintenance logbook until the end of the calendar year, when they are archived electronically.

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**3.6 Thermo-Mixer**

(critical instrument)

**3.6.1 Operation**

- Turn on main switch.
- Calibrated set points for the digital display in the thermomixer unit are checked semi-annually and noted on the instrument, with analyst date and initials. Choose digital temperature set point accordingly.
- Allow thermomixer to come to temperature, as shown on the digital display.

**3.6.2 Maintenance**

- As a bi-annual performance check, the digital set-points for specified temperatures (i.e. 56°C and 70°C) will be re-assessed.

Procedure:

- Put ~1000uL of sterile water in a tube and place in the thermomixer (without shaking).
- Set temperature on the digital display to the previously calibrated value noted on the instrument. Allow thermomixer to come to temperature, as shown on the digital display.
- Use a temperature probe to determine actual temperature.

Evaluating results:

- If the digital set point is no longer correct but within +/- 5 °C of the temperature displayed on the screen, adjust gradually until the correct set-point is found, allowing adequate time for temperature stabilization during the adjustment process.

Documenting completion and approval / rejection:

- Note any changes to the digital set-points on the instrument.
- Completion is documented on the Thermomixer Temperature Log.

Unacceptable results:

- If the temperature on the thermometer and the displayed temperature differ by more than 5 °C, or if the temperature reading will not stabilize, the instrument will be taken offline – label the instrument clearly as offline, note as offline in the maintenance log, and notify the Technical Leader.

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**3.7 Applied Biosystems 7500 Real-Time PCR System (critical instrument)**

*Reference: ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System User Bulletin*  
*Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*

Maintenance Procedures

Directions for performing the checks listed below are located in *ABI Prism 7000 Sequence Detection and Applied Biosystems 7500 Real Time PCR System Maintenance Guide*. Weekly maintenance is recorded in the Maintenance Log (located at the end of this document). Monthly and semi-annual maintenance is recorded on the monthly and semi-annual maintenance form. **Note:** it is not necessary to perform maintenance if the instrument is not in use for the relevant time period. Record as "Not In Use" in the Maintenance Log as applicable.

Daily in use

- Power off the computer controlling the 7500 instrument, then after 30 seconds, power on the computer.
- Clean the surface of the 7500 instrument with a lint-free cloth.

Monthly

- Check the lamp status. If necessary, replace the halogen lamp.
- Perform a background calibration
- Run disk cleanup and disk defragmentation.

Semiannually:

- Perform a regions of interest (ROI) calibration
- Perform a background calibration.
- Perform an optical calibration.
- Perform a dye calibration. At a minimum these dyes must be calibrated: VIC, FAM, ABY, JUN, and Mustang Purple.
- Perform an RNase P instrument verification run.

Annually:

- Preventive maintenance (performed by vendor, see paragraph below). Note: some or all semiannual maintenance tasks may be covered as part of the annual preventive maintenance.

As needed

- Decontaminate the 7500 instrument
- Replace the halogen lamp
- Replace the 7500 instrument fuses
- Update the Windows operating system
- Update the 7500 software.

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- Check computer disk space. If necessary, archive experiment files.

Annual preventive maintenance (PM): This is conducted and evaluated by an outside vendor, according to their protocols. It includes temperature verification, ROI / background, and optical calibrations, dye calibrations, and other functionality tests. This serves as the annual performance check of those systems. Documentation consists of a completed report from the vendor technician, which is reviewed to confirm that results were passing. The report is added to the instrument maintenance logbook until it is archived electronically at the end of the calendar year.

Performance Check following annual PM

Following annual preventive maintenance or service/repairs, a performance check will be run prior to using the instrument for casework or database analysis. Typically, this is run by an analyst or technician. However, it may be run by an outside vendor, if it is documented that the performance check happened *\*after\** any/all repairs or adjustments to the instrument.

Procedure:

- A standard curve and NTC wells are run according to the procedure in the current version of the Forensic Biology Procedure Manual (FBPM).
- Since the RNase P plate contains these elements, a successful RNase P plate run can also serve as a performance check.

Evaluation:

- This data will be evaluated using the defined quality metrics from the FBPM.

Unacceptable data:

- If any of the quality metrics do not fall in the acceptable range defined in the FBPM, the performance check should be repeated with a new plate.
- If the plate fails a second time, the instrument must be noted as offline, both on the instrument itself and in its logbook, and the Technical Leader must be notified.

Documenting completion and approval/rejection:

- A printout of the report and the reagent worksheet is added to the Maintenance Log binder.
- The PC pass section is completed on the maintenance log, along with the date of completion.

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**3.8 Applied Biosystems ProFlex Thermal Cyclers (critical instrument)**

*Reference: Applied Biosystems ProFlex® PCR System User Guide*  
[ProFlex PCR System User Guide \(Pub. no. MAN0007697\) \(thermofisher.com\)](#)

Maintenance Procedures

The maintenance log form is provided at the back of this document.

Monthly maintenance: During the last week of each month (+/- one week), the wells and cover should be cleaned, and a self-verification test run. Maintenance is recorded on the maintenance log.

- Before cleaning, power off the instrument by disconnecting the power. Allow the instrument to cool until the heated cover and sample block reach room temperature.
- To clean the sample wells, remove the sample tray from the sample block and set it aside. Use a cotton swab soaked in isopropanol to clean the sample wells thoroughly. Make certain the isopropanol has evaporated completely before reloading a sample tray.
- To clean the heated cover, soak a cotton swab or piece of clean cloth with isopropanol and gently wipe the heated platen. Blot off any remaining isopropanol from the cover and make certain the isopropanol has evaporated completely before restarting the instrument.
- To run a self-verification test:
  - From the Home screen, touch Settings.
  - In the Settings screen, touch Maintenance & Services.
  - In the Maintenance & Settings screen, touch Self verification Test.
  - In the Self verification test screen, touch Start Test to begin testing. Test takes about ten minutes. Once the test is completed the test results will be displayed in the form of a report.
  - Note passing test in Maintenance Log.
    - It is not necessary to save the Self Verification Test report unless the test does not pass.
    - If the Self Verification Test does not pass on two attempts, mark the instrument as offline and notify the DNA Technical Manager to determine a course of action.

Preventive maintenance and other service:

- Annual temperature measurement and verification are performed by a vendor representative. Records are retained and archived annually.
- Annual preventive maintenance (PM) and any service to the instrument, as well as the dates that an instrument is taken out of service or returned to service, are recorded in the maintenance log.
- Following annual preventive maintenance or any service and prior to being used again for casework/database analyses, the instrument must
  - successfully pass a self-verification test

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- successfully pass a performance check, consisting of amplification and analysis of a positive and negative amplification control. This may be performed using any one of the amplification kits currently in use at AK SCDL. Controls are evaluated using criteria defined in the Forensic Biology Procedure Manual. Documentation consists of a printout of the positive and negative controls and marking PC following PM/service in the maintenance log.

As needed:

- Clean the touchscreen with any commercially available LCD cleaning product, being careful not to scratch the screen.
- If sample wells or heated cover become contaminated, clean thoroughly with a cotton swab soaked in 1:10 solution of Clorox bleach, then rinse with water.

**3.9 Applied Biosystems 3500xl (critical instrument)**

*References: Applied Biosystems 3500xl Genetic Analyzers User Guide*

[http://www3.appliedbiosystems.com/cms/groups/mcb\\_support/documents/generaldocuments/cms\\_104815.pdf](http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_104815.pdf)

Annual Preventive Maintenance is performed in-house by manufacturer personnel. The maintenance is recorded on the maintenance log in a binder near the instruments. The service report is also maintained with the instrument records. Additional maintenance, also recorded in the log, is described below. Instrument maintenance records are archived in SharePoint annually.

**3.9.1 Maintenance to be performed as needed**

- Ensure adequate levels of buffer in reservoirs
- Purge old plate records
  - Click **Library** and select **Plates** in the navigation pane. All plates stored within the library will appear on the screen.
  - Select the plates to be deleted (more than one can be selected at a time).
  - Right click the mouse and select **delete**.

**Note:** Do not use the purge feature to delete items in the library. Doing so will delete all items with the exception of factory stored items. Thus, all multiplex assays and protocols from other manufacturers will be deleted.

**3.9.1.1 Replacing Anode Buffer Container (ABC)**

The Anode Buffer Container (ABC) must be replaced after 14 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.

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- Ensure that most of the 1X buffer is in the larger side of the ABC container prior to removing the seal by tilting the container slightly.
- Place the ABC into the Anode end of the instrument, below the pump. (RFID tag will face the instrument).

### 3.9.1.2 Replacing Cathode Buffer Container (CBC)

The Cathode Buffer Container (CBC) must be replaced after 14 days or 50 injections.

- Allow buffer container to equilibrate to room temperature prior to placing on the instrument.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Wipe away any condensation on the exterior of the CBC using lint free lab cloth.
- Tilt the CBC back and forth gently to ensure the buffer is evenly distributed and remove the seal.
- Ensure the top of the CBC is dry (failure to do this may result in arcing) and place the appropriate septa on both sides of the CBC.
- Install the CBC on the autosampler.

### 3.9.1.3 Replenishing Polymer

The polymer must be replaced after 960 samples (or 120 injections) or when it has passed the expiration date.

- Click **Maintenance** (top right of the screen). In the Maintenance Wizards screen, click **Replenish Polymer** (this will take 10 to 20 minutes to complete) and follow the prompts.
- Polymer may be replenished as part of the water wash wizard.

### 3.9.1.4 Replacing the Capillary Array

The capillary is replaced as needed; when indicated by poor data quality.

- The following indications may suggest that a new capillary array is required:
  - Poor sizing precision or allele calling
  - Poor resolution and/or decreased signal intensity
- In the Maintenance Wizards screen click **Install Capillary Array** (this will take 15-45 minutes to complete) and follow the prompts.

**Note:** Spatial and Spectral Calibrations must be performed anytime an array is replaced. A water wash, water trap flush and performance check must also be completed to verify performance of the array.

### 3.9.1.5 Spatial Calibration

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A spatial calibration establishes a relationship between the signal emitted by each capillary and the position where that signal falls on and is detected by the CCD camera. A spatial calibration must be performed when the capillary array has been replaced, the detector door has been opened, or the instrument has been moved. **Note:** A spatial is performed during the Performance Check procedure and does not need to be run separately if the performance check procedure is run.

Performing a Spatial Calibration

- Access the Spatial Calibration screen:
  - Click **Maintenance** and then select **Spatial Calibration** in the navigation pane.
- Under Options, select **NO-Fill** or select **Fill** to fill the array with polymer before starting the calibration.
- Select **Perform QC Checks** to enable the system to check each capillary against the specified range for spacing and intensity.
- Click **Start Calibration**.

Evaluating a Spatial Calibration

- Evaluate the spatial calibration profile to ensure that you see:
  - One sharp peak for each capillary. Small shoulders are acceptable
  - One marker (+) at the top of every peak.
  - Peaks are about the same height.
- If the results meet the above criteria, click Accept Results. If the results do not meet the above criteria, click Reject Results and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide, “Spatial calibration troubleshooting” page 300.
- If the results are acceptable, a printed copy of the passing spatial calibration report is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.

**3.9.1.6 Spectral Calibration**

A spectral calibration creates a de-convolution matrix that compensates for dye overlap. A spectral calibration should be performed for each chemistry used whenever the capillary array is changed, the CCD camera or laser are realigned or replaced, following preventative maintenance that affects the optical components, or if you see a decrease in spectral separation.

Performing a Spectral Calibration – Dye Set J6 (for use with Global Filer and capillary performance checks) and J6-OSR (for use with Global Filer Express)

- In the Dashboard, Click **Start Pre-heat 60°** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.

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- Thoroughly mix the contents of the DS-36 Matrix Standard (Dye Set J6) tube and spin briefly in a microcentrifuge.
- Prepare the matrix standard by combining the following in a 1.5 mL microcentrifuge tube:
  - For J6 (or J6-OSR) matrix: Standard: 6 µL and Hi-Di Formamide: 294 µL
- Dispense 10 µL of the matrix standard/Hi-Di formamide mixture into the first 24 wells (three columns) of a 96 well CE plate and cover with plate septa.
- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Denature at 95°C for 5 minutes. Snap chill for three minutes.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
  - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **J6** (or **J6-OSR**) as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.
- Note: The same spectral plate will need to be run two times: once with J6 and once with J6-OSR.

Performing a Spectral Calibration – Dye Set Promega G5 (for use with PowerPlex Y23)

- In the Dashboard, Click **Start Pre-heat 60°** at least 30 minutes prior to the start of the run.
- Ensure the consumables are not expired and adequate injections remain.
- Ensure the pump assembly is free of bubbles, run the Remove bubble wizard if needed.
- At first use, thaw the 5C Matrix Mix and Matrix Dilution Buffer completely. After first use, store reagents at 2 – 10 C, protected from light.
- Vortex the Matrix Mix for 10 – 15 seconds. Add 10 µL of 5C Matrix Mix to one tube of Matrix Dilution Buffer. Vortex for 10-15 seconds. Note date of dilution on side of tube. Diluted Matrix Mix can be stored up to one week at 2 – 10 C.
- Add 10 µL of the diluted 5C Matrix Mix to 500 µL of Hi-Di formamide. Vortex for 10-15 seconds.

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- Dispense 15µL of the master mix into the first 24 wells (3 columns) of a 96 well CE plate and cover with a plate septa. **DO NOT HEAT DENATURE.**
- Briefly centrifuge the plate containing the standards and verify that each sample does not contain bubbles and is positioned correctly in the bottom of the well.
- Place the sample plate into the plate base provided with the instrument.
- Snap the plate cover onto the plate, septa, and plate base.
- Verify that the holes of the plate retainer and the septa are aligned.
- Press the tray button on the instrument to bring the autosampler to the forward position.
- Place the plate in the autosampler with the labels facing you and the notched corner of the plate in the notched corner of the autosampler. Close the instrument doors.
- Access the Spectral Calibration screen:
  - Select **Maintenance**, then click **Spectral Calibration** in the navigation pane.
- Select **96** for the number of wells in the spectral calibration plate and specify the plate location (A or B) in the instrument.
- Select **Matrix Standard** as the chemistry standard and **Promega G5** as the dye set.
- Select **Allow Borrowing**.
- Click **Start Run**.

Evaluating a Spectral Calibration

- Passing and failing capillaries are shown in green and red respectively. Borrowed capillaries are shown in yellow with an arrow indicating the adjacent capillary from which results were borrowed. Up to three adjacent-capillary borrowing events are allowed.
- If fewer than the recommended number of capillaries pass, the spectral calibration run will be repeated automatically up to three times.
- View the raw data for each capillary. Ensure that the data meet the following criteria:
  - Order of the peaks in the raw data profile from left to right is orange-red-yellow-green-blue for G5 and Promega G5 Dye Sets, and , orange-red-yellow-green-blue-purple for Dye Set J6.
  - The Quality Value is  $\geq 0.95$  and the Condition Number is  $\leq 8.0$  for J6; the Quality Value is  $\geq 0.95$  and the Condition Number is  $\leq 13.5$  for G5 and Promega G5.
- If the data for all capillaries meet the above criteria, click **Accept Results**.
- If any capillary data does not meet the criteria click **Reject Results** and refer to the Applied Biosystems 3500/3500xl Genetic Analyzer User guide "Spectral calibration troubleshooting" page 301.
- If the results are acceptable, a printed copy of the passing spectral report is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF**

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**Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.

### 3.9.2 Daily In Use Maintenance

The computer and instrument are restarted and wiped with sterile water on a Kimwipe.

### 3.9.3 Monthly Maintenance

The water wash and water trap flush are performed as part of monthly maintenance and/or anytime an array is replaced.

#### 3.9.3.1 Computer maintenance

- Defragment the hard drive  
**Start > Programs > Accessories > System Tools > Disk Defragmenter**

#### 3.9.3.2 Water Wash

- The water wash may take over 40 minutes to complete
- Click **Maintenance** (top left of screen) on the dashboard.
- Select Wash Pump and Channels to run the wizard. Follow the prompts to completion.

**Note:** An empty ABC reservoir may be used instead of emptying the reservoir currently on the instrument. Simply remove from the instrument, cover, and set aside. At the completion of the Water Wash Wizard, replace the ABC with the reservoir previously removed from the instrument or a new reservoir.

#### 3.9.3.3 Water Trap Flush

- Fill the supplied 20ml Luer lock syringe with warm deionized water. Expel any bubbles from the syringe.
- Attach the syringe to the forward-facing Luer fitting at the top of the pump block. Hold the fitting with one hand while threading the syringe clockwise.
- Open the Luer fitting by grasping the body of the fitting and turning it counterclockwise approximately one-half turn to loosen.
- Flush 5ml of deionized water through the trap taking extra care not to use excessive force.
- Remove the syringe from the Luer fitting by holding the fitting with one hand while turning the syringe counterclockwise.
- Close the Luer fitting by lightly turning clockwise until the fitting seals against the block.
- Empty the water trap waste container.

#### 3.9.3.4 Monthly Maintenance for Offline 3500 Instruments

- Follow the Water Trap Flush procedure described above
- Use a syringe with tubing attached where the polymer would be attached to flush the pump and channels with deionized water

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- Ensure that the liquid level in the ABC container is full

### 3.9.4 Performance Check

A performance check provides for assessment of the instrument system's resolution and its ability to adequately resolve the peaks of an allelic ladder within one base pair. Also, it monitors the ability of the instrument to produce consistent peak heights over the relevant sizing range.

This performance check is performed at a minimum every three months. This performance check is also performed after capillary array changes and after PM or service has been performed on a 3500 instrument prior to resuming use for casework or database analysis.

#### Procedure:

- Follow the instructions in the Forensic Biology Casework Procedures Manual to prepare the 3500xl for a run
- If the capillary has been changed, follow the manufacturer's instructions to run a DS-36 (dye set J6) spectral calibration.
- Prepare an allelic ladder master mix by adding the following volumes of reagents to an appropriately sized tube:
  - 12µl GeneScan 600 LIZ
  - 30ul GlobalFiler allelic ladder
  - 288µl Hi-Di Formamide
- Vortex the master mix and spin briefly. Transfer 10µl of the master mix to the appropriate wells (i.e. A1-H3). Briefly centrifuge the plate, then heat and snap chill the plate. Prepare plate assembly and load on the 3500.
- To access the Fragment Install Standard screen: Select **Maintenance**, then select **HID Install Standard** in the navigation pane.
- Select the plate type and plate position in the instrument. Note: you do not create a plate for the performance check – the software uses predetermined positions for the run.
- Click **Start Run**. The run takes about 30 minutes.
- The software evaluates data for all capillaries, including:
  - Nominal allele sizes for all markers
  - Average peak heights
  - Sizing precision
  - Pass/fail

#### Evaluating results:

- Evaluate the standard data as follows:
  - Examine the number of size standard and allele peaks found for each capillary. The number of expected peaks is shown above the observed number of peaks for each of the capillaries.
  - If the expected number of alleles and size standard peaks are found, click Accept Results.

#### Unacceptable data:

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- If the expected number of alleles and size standard peaks are not found, click Reject results. Rerun the plate. If the check is still not successful, consult the 3500 User Guide for troubleshooting suggestions. If the instrument still does not pass, consult the DNA Technical Manager and notify discipline scientists that the instrument is offline until the issue is resolved. Notification includes marking the instrument itself as offline and adding a note to the maintenance logbook.

## Documentation of completion and approval/rejection

- A printed copy of the passing performance check is placed in the 3500 Maintenance Logbook. To print a copy of the report, click **View Detail Report**, then click **Print**. In the printer dialog box, select **CutePDF Writer** as the printer. Save this to a thumb drive, which can then be printed from a computer that is networked to a printer.
  - Since performance checks need to occur every three months, after the completion of the performance check, the next due date will be set for the same day of the month, three months later. For example, if a performance check was completed on February 1, the due date for starting the next performance check would be May 1.

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**3.10 Pipettes****(critical instrument)**

Pipette calibration records are tracked in SharePoint.

**Performance check**Procedure and evaluation:

- Pipettes must be bleached before and after calibration.
- Pipettes are calibrated annually in-house by a suitable vendor, using their own procedure and evaluation, for the specified volume range of the pipette.

Unacceptable data:

- Pipettes identified by the vendor as not passing are labelled as offline and removed from lab workspace.

Documentation of completion and approval/rejection:

- ISO certificates of calibration received from the vendor are archived in SharePoint.
- Certificates indicate pipettes as passing or failing.
- Individual pipettes are each labeled with the calibration date and the next due date for calibration.

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**3.11 Thermometers****(critical instrument)****Non-electronic thermometers and probes used for extraction lab instrument performance checks**

Procedure and evaluation: These are purchased as needed and can be used without performance checks until the expiration of their certificate of calibration.

Unacceptable data: Broken or otherwise non-functional thermometers are discarded and replaced.

Documentation: Non-electronic thermometers are tracked on an uncontrolled spreadsheet. The spreadsheet includes, for each thermometer, the date of calibration, in-service date, and date due for replacement. New thermometers are labelled with the date of expiration of their certificate of calibration.

**3.12 QIAcube®****(critical instrument)**

*References: QIAcube® User Manual, Version 1.1, 06/2008*

**3.12.1 Regular maintenance procedure**

After running a protocol, perform the regular maintenance procedure:

- Wipe down platform with a kimwipe moistened with ethanol and then distilled water.
  - Do not directly spray the inside of the QIAcube with water or ethanol!
- Empty the waste drawer.
  - If necessary, wipe down with a kimwipe moistened with ethanol and then distilled water.
- Remove used disposable labware and unwanted samples and reagents from the worktable. Discard in biohazardous waste.
  - Plastic rotor adaptors are single use only
- Replace the lids of the reagent bottles and close tightly.
- Rerack tips if there are any partially used racks.

**3.12.2 Monthly Maintenance Procedure**

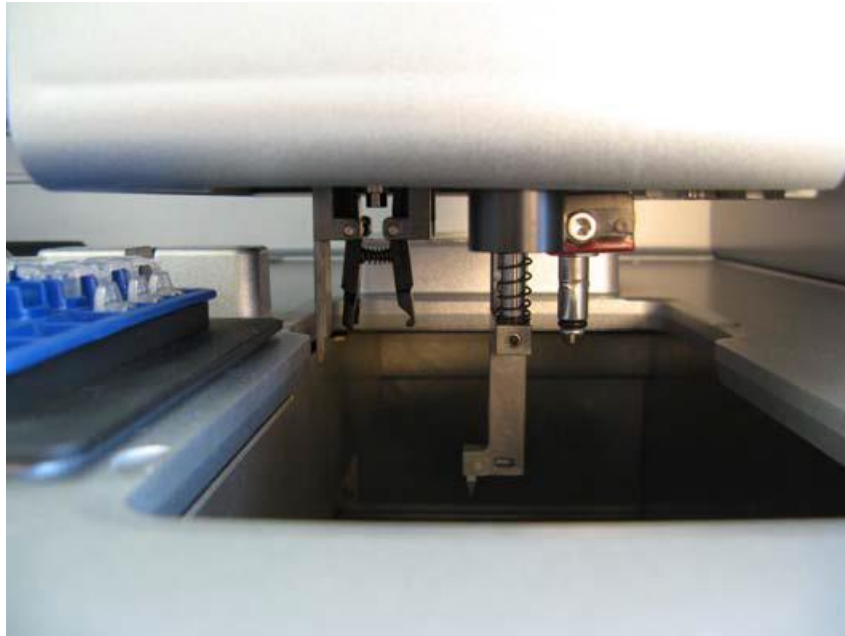
- Clean the optical sensor, tip adapter, gripper unit (including the gripper), the stabilizing rod, and the spin column lid holder, by wiping these modules with a soft lint-free cloth moistened with water.
  - To gain access to the modules within the robotic arm:
    - “Tools” => “Maintenance” => “Cleaning position”

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- Be sure to remove the waste drawer and the labware tray to prevent robotic arm from crashing into tray.
- Visually and manually inspect the O-ring to make sure it is intact (not cracked and seated properly).



- Wipe down the following with a kimwipe moistened with ethanol and then distilled water. Wipe dry.
  - Worktable
  - Underneath centrifuge rotor
  - Centrifuge, centrifuge gasket, and centrifuge lid
  - Shaker rack, labware tray, heating adapter, reagent bottle rack, rotor plastic holder
  - Waste drawer liner (and drawer, if needed)
- Wipe the inside and outside of the QIAcube with distilled water.
  - Do not use alcohol or alcohol-based disinfectants on the QIAcube door.
  - Wipe the touchscreen with a kimwipe moistened with ethanol and then distilled water. Wipe dry with a paper towel.
- Perform regular maintenance procedure but remove reagent bottles from QIAcube.
- Perform a Tightness Test
  - The tightness test is performed to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.
  - Load an empty 2 ml microcentrifuge tube in position 1 of the shaker.
  - Fill a reagent bottle with reagent alcohol and place in position 1 of the reagent bottle rack.
  - Load a tip rack of 1000 µl wide-bore filter tips onto the QIAcube.

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- Start Tightness Test
  - In the main menu, press “Tools”.
  - Select “Maintenance”
  - Select “Tightness test”
  - Select the appropriate type of filter-tips (“1000 µl wide-bore tips”)
  - Press “Start” to start the tightness test with the selected type of filter-tips.
  - Follow the instructions displayed in the touchscreen, and press “Start” to start the tightness test.
- After the load check, the robotic arm will pick up a tip, aspirate ethanol, and move to the tube. The tip will remain in place above the tube for 2 minutes. The tip will be detached.
- After the protocol is completed, open the QIAcube door and check if the tube contains liquid.
  - PASS: If the tube is still empty and dry, the tightness of the pipetting system is adequate, and the test result is passing.
  - FAIL: Liquid present in the tube at the end of the test indicates a failure of the test. If you find liquid in the tube, change the O-ring and repeat the test. If the second test fails, the instrument must be taken offline until the issue is resolved. Note the test results in the maintenance documentation and put a note on the instrument to show that it is offline. Notify the technical manager.
- Based on results of tightness test, if necessary, change O-ring (see QIAcube® Tip-Adapter Ring Replacement protocol). NOTE: The ring should be changed at least annually. O-ring change is noted in the maintenance log.

### 3.12.3 Bi-yearly Maintenance Procedure

- Perform monthly maintenance procedure
- Access to the inside of the centrifuge is required. Lid should be open provided that a protocol is not being run. In case it is closed, to open centrifuge lid:
  - “Tools” => “Maintenance” => “Open lid”
- Switch off the QIAcube at the power switch.
- Remove the buckets from the rotor. Undo the rotor nut on rotor top using the rotor key and lift the rotor off the rotor shaft.
- Rinse the rotor, buckets, and rotor nut in ethanol then distilled water. Use a swab to reach narrow areas. Wipe surfaces dry with a soft lint-free cloth.
- Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) on a soft, lint-free cloth, and wipe the bucket mount and rotor claw. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent.
  - Important: Before applying oil to the rotor buckets on the rotor, make sure that the rotor and all buckets are completely dry.

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- Clean the inside of the centrifuge, centrifuge gasket, and centrifuge lid with ethanol then distilled water. Wipe dry with lint-free paper towel.
- Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.
- Reinstall rotor and buckets
  - The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position
    - Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft. Install the rotor nut on top of the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated.
  - When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely.
    - Important: All centrifuge buckets must be mounted before starting a run.

#### 3.12.4 Bi-annual Performance Check

Approximately once every six months, as well as after repair or service, a performance check will be run on each QIAcube instrument.

Procedure: The performance check will consist of one known sample (including both sperm and epithelial cells), and one reagent blank sample. Performance check samples will be taken through the casework protocol for differential extraction with automated wash protocol.

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Evaluation: Extracts generated in the performance check are quantified, amplified, and analyzed. Resulting data is analyzed according to criteria described in the current version of the Forensic Biology Procedure Manual. In addition, they are checked to make sure the correct profiles were obtained. A passing performance check consists of correct and complete profiles for both the sperm and epithelial fractions of the positive control sample, plus amplified reagent blank profiles without extraneous DNA.

Unacceptable data: A performance check failure would be an incomplete or incorrect positive control profile, or a reagent blank profile with detected DNA. However, an incomplete profile, and some instances of reagent blank contamination, may be indicative of an issue at the amplification stage. Such samples should be re-amplified. If re-amplification does not yield a full, correct profile for a positive control sample and/or a reagent blank profile without extraneous DNA, then the performance check fails. The instrument must be taken offline and the Technical Manager must be notified, so that a further course of action can be determined by the Technical Manager.

Documentation of completion and approval/rejection: Performance check documentation includes electropherograms of the successful positive and negative controls. If the performance check was performed as a part of a casework central log, the central log may be referenced. If the performance check was performed alone, documentation such as allelic ladders, amplification controls, etc. should be included with the performance check paperwork. An electronic scan of the compiled performance checks documentation for a calendar year is then stored on the laboratory network at the end of the year.

### 3.13 Heat block for capillary electrophoresis plate prep (critical instrument)

The heat blocks hold up to two plates. They are always left on and set to ~97°C, and performance is monitored before each use by checking the NIST-traceable thermometer in the corresponding side of the heat block.

#### Performance check

Procedure and evaluation: Prior to putting a plate in the heat block, analyst will check that the thermometer temperature for that side of the heat block is in acceptable range (95 to 99°C).

Unacceptable data: If the thermometer indicates that the heat block side is not within the acceptable temperature range, the analyst will mark the side as offline on the instrument and on the log, and notify the Technical Leader.

Documentation of completion and approval/rejection: Analyst will complete the log for each use; a thermometer temperature in range is documentation of a passing result.



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**DNA Critical Reagent Verification Form****Scientist:****Date:****3500 data folder:****Central log (if applicable):****Lot #****Expiration Date****Buffer ATL****Buffer MTL****DTT****EZ1 Kits**

EZ1 Reagent Cartridges

Proteinase K

G2 Buffer

Carrier RNA

**G2 Buffer****GlobalFiler Express Kit**

DNA Control 007

Master Mix

Master Mix Additive

Primer Set

GlobalFiler Express Allelic Ladder

**Prep-N-Go Buffer****Proteinase K****Sterile Water****TE<sup>-4</sup> Buffer**

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**Quantifiler Trio Verification Form****Scientist:****Date:**

New Kit Lot# \_\_\_\_\_

Expiration Date: \_\_\_\_\_

PCR Reaction Mix: \_\_\_\_\_

Primer: \_\_\_\_\_

DNA Standard: \_\_\_\_\_

Dilution Buffer: \_\_\_\_\_

PREVIOUS QT Y-intercept values

T-Large Autosomal: \_\_\_\_\_ T-Small Autosomal \_\_\_\_\_: T-Y \_\_\_\_\_

NEW QT Y-intercept values

T-Large Autosomal: \_\_\_\_\_ T-Small Autosomal \_\_\_\_\_: T-Y \_\_\_\_\_

1. Set up and run a standard curve and two NTC wells with the new kit reagents.
2. Confirm that passive references values are acceptable and note as such on front page of Experimental Results Report.
3. Passing value for  $R^2$  is  $\geq 0.98$ . Passing value for slopes is -3.0 to -3.6. If either of these results is outside range, run a new standard curve and two NTC wells. If second attempt also fails, notify the DNA TM.
4. Passing values for Y-intercepts are 25.8 – 26.3 for the male standard curve, 24.9 – 25.6 for the large autosomal human standard curve, and 26.8 – 27.3 for the small autosomal human standard curve. If any of these results are outside range, run a new standard curve and two NTC wells. If second attempt also fails, notify the DNA TM.
5. Provide this sheet and the Experimental Results Report (to include the summary page, plate layout, human and male standard curves, and results table) to the DNA TM.

DNA Technical Manager

Kit is acceptable for use:

Conditions / observations / notes:

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## GlobalFiler Verification

**Scientist:****Date:**

Identity of known sample used for verification: \_\_\_\_\_

**OLD Kit**                      **Lot #** \_\_\_\_\_ **Expiration Date:** \_\_\_\_\_**NEW Kit**                      **Lot#** \_\_\_\_\_ **Expiration Date:** \_\_\_\_\_

DNA Control 007                      Allelic Ladder \_\_\_\_\_

Primer Set                      Master Mix \_\_\_\_\_

1. Amplify the positive control, a negative TE<sup>-4</sup> control, and a known sample extract using the kit currently in use (OLD kit). The positive and negative from the old kit are assessed only to ensure that the old reagents are working correctly for amplification for the known sample.
2. Amplify the positive control, a negative TE<sup>-4</sup> control, and the same known sample extract using the kit to be validated (NEW kit).
3. Run all samples using the Allelic Ladder from the kit to be validated (NEW kit).
4. Assess peak height variation:
  - a. Export peak heights and allele calls for both the known sample extract run with the OLD kit and the same known sample extract run with the NEW kit.
  - b. Calculate average peak height for each kit.
5. Print the following and submit to DNA TM along with this page:
  - a. Amplification worksheet (can write "see verification worksheet" in reagent section)
  - b. Positive control (NEW kit)
  - c. Negative control (NEW kit)
  - d. Allelic Ladder (NEW kit)
  - e. Known sample (NEW kit)
  - f. Known sample (OLD kit)
  - g. Average Peak Height assessment (as described in step 4)

DNA Technical Manager

Validation range of peak heights for single source sample is 11,881 RFU – 3850 RFU.

New kit lot is within range of validation? \_\_\_\_\_

30% Range from OLD kit: \_\_\_\_\_ NEW kit within 30%? \_\_\_\_\_

Kit is acceptable for use:

Conditions / Observations / Notes:

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**PowerPlex Y23 Verification****Scientist:****Date:**

Identity of known sample used for verification: \_\_\_\_\_

**OLD Kit**                      **Lot #** \_\_\_\_\_ **Expiration Date:** \_\_\_\_\_**NEW Kit**                      **Lot#** \_\_\_\_\_ **Expiration Date:** \_\_\_\_\_

DNA Control 2800                      Allelic Ladder \_\_\_\_\_

Primer Set                      Master Mix \_\_\_\_\_

ILS WEN 500 Y23 \_\_\_\_\_

1. Amplify the positive control, a negative TE<sup>-4</sup> control, and a known sample extract using the kit currently in use (OLD kit). The positive and negative from the old kit are assessed only to ensure that the old reagents are working correctly for amplification for the known sample.
2. Amplify the positive control, a negative TE<sup>-4</sup> control, and the same known sample extract using the kit to be validated (NEW kit).
3. Run all samples using the Allelic Ladder from the kit to be validated (NEW kit).
4. Assess peak height variation:
  - a. Export peak heights and allele calls for both the known sample extract run with the OLD kit and the same known sample extract run with the NEW kit.
  - b. Calculate average peak height for each kit.
5. Print the following and submit to DNA TM along with this page:
  - a. Amplification worksheet (can write "see verification worksheet" in reagent section)
  - b. Positive control (NEW kit)
  - c. Negative control (NEW kit)
  - d. Allelic Ladder (NEW kit)
  - e. Known sample (NEW kit)
  - f. Known sample (OLD kit)
  - g. Average Peak Height assessment (as described in step 4)

\_\_\_\_\_  
DNA Technical Manager

Validation range of peak heights for single source sample is 9722 RFU – 1925 RFU.

New kit lot is within range of validation? \_\_\_\_\_

30% Range from OLD kit: \_\_\_\_\_ NEW kit within 30%? \_\_\_\_\_

Kit is acceptable for use:

Conditions / Observations / Notes:

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**GenTegra-DNA Verification**

Scientist:

Date:

Identity of known sample used for verification: \_\_\_\_\_

GenTegra-DNA Lot# \_\_\_\_\_ Expiration Date on package: \_\_\_\_\_

Expiration for Hydrated 5X stock solution (3 months after prep date): \_\_\_\_\_

1. Use GenTegra-DNA (GTD) to dry down a previously typed reference sample extract (example: 50  $\mu$ L) and a corresponding reagent blank extract (example: 50  $\mu$ L). Note: the volume used does not have to be 50  $\mu$ L, but it must be at least 20  $\mu$ L and must be consistent between the sample and the blank.
2. Rehydrate each of the extracts back to its previous volume (example: 50  $\mu$ L) and amplify, using the same amplification volumes previously used.
3. Confirm that the reagent blank is clean, using interpretation criteria from the current version of the Forensic Biology Procedure Manual.
4. Assess peak height variation in the known sample:
  - a. Export peak heights and allele calls for both the known sample extract amplified PRIOR to GenTegra-DNA, and the same known sample extract amplified AFTER GenTegra-DNA dry-down and re-hydration.
  - b. Calculate average peak height for each amplification of the reference sample extract.
  - c. Comparison of the original amplification results to the results of the rehydrated extract should not demonstrate a significant (>30%) overall reduction in the average peak heights.
5. Documentation in SharePoint: Scanned printout of the reagent blank electropherogram (showing primer peak) and the newly typed reference sample extract, and Peak Height Assessment (see following page for example), along with this form.

Conditions / Observations / Notes:

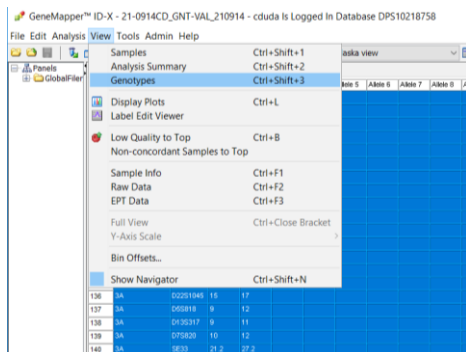
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**How to calculate average heterozygous peak heights for verification**

1. Open project containing “before” sample in GeneMapper ID-X
2. Ensure that all artifacts are struck in the profile of interest
3. Under the View tab, select Genotypes:



4. Copy and paste sample name, markers, alleles, and peak heights for the “before” sample into an Excel spreadsheet.
5. Repeat for “after” sample, adding the data into the same Excel spreadsheet, a few rows below the “before” sample data.
6. Delete blank columns
7. Calculate the sum of the peak heights for the “before” sample. In the example below, the formula would read =SUM(E1:F24)
8. Calculate the average heterozygous peak height for the sample by dividing the summed peak heights by either 46 (if the profile is male) or 44 (if the profile is female). In the example below, the formula would read =F25/46
9. Repeat for “after” sample.
10. Calculate acceptable range by multiplying original peak height by 30% (or other percentage as needed). In the example below,  $3413 * 0.30 = 1023$ . Therefore, the range within 30% is (3413-1023) to (3413+1023), or 2390 to 4436.
11. Ensure that the “after” average peak height is within specified range.
12. See next page for an example of complete documentation.

	A	B	C	D	E	F	G	H
1	3A	D3S1358	17	18	5949	4678		
2	3A	vWA	17	18	2686	1974		
3	3A	D16S539	11	12	2279	1923		
4	3A	CSF1PO	10		2957			
5	3A	TPOX	8		2312			
6	3A	Yindel	2		7006			
7	3A	AMEL	X	Y	6008	5056		
8	3A	D8S1179	10	14	6106	6583		
9	3A	D21S11	30	32.2	2188	1988		
10	3A	D18S51	16	18	2376	2153		
11	3A	DYS391	10		2482			
12	3A	D2S441	11.3	14	5837	4758		
13	3A	D19S433	13	15	4362	4417		
14	3A	TH01	7	9	3791	3818		
15	3A	FGA	18	25	2605	2533		
16	3A	D22S1045	15	17	4161	4249		
17	3A	D5S818	9	12	4213	3386		
18	3A	D13S317	9	11	3360	3233		
19	3A	D7S820	10	12	2996	2126		
20	3A	SE33	21.2	27.2	2445	1756		
21	3A	D10S1248	13		9544			
22	3A	D1S1656	12	17.3	3577	3409		
23	3A	D12S391	17	19	2626	2704		
24	3A	D2S1338	17	21	1928	2446		
25					Sum of peak hts:	158984		
26					Average (sum/46):	3413		
27								

**Example of Peak Height Assessment for GenTegra-DNA/GF/PPY23 Kit Verification**

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Sample Name	Marker	Allele 1	Allele 2	Height 1	Height 2
3A-before	D3S1358	17	18	5949	4678
3A-before	vWA	17	18	2686	1974
3A-before	D16S539	11	12	2279	1923
3A-before	CSF1PO	10		2957	
3A-before	TPOX	8		2312	
3A-before	Yindel	2		7006	
3A-before	AMEL	X	Y	6008	5056
3A-before	D8S1179	10	14	6106	6583
3A-before	D21S11	30	32.2	2188	1988
3A-before	D18S51	16	18	2376	2153
3A-before	DYS391	10		2482	
3A-before	D2S441	11.3	14	5837	4758
3A-before	D19S433	13	15	4362	4417
3A-before	TH01	7	9	3791	3818
3A-before	FGA	18	25	2605	2533
3A-before	D22S1045	15	17	4161	4249
3A-before	D5S818	9	12	4213	3386
3A-before	D13S317	9	11	3360	3233
3A-before	D7S820	10	12	2996	2126
3A-before	SE33	21.2	27.2	2445	1756
3A-before	D10S1248	13		9544	
3A-before	D1S1656	12	17.3	3577	3409
3A-before	D12S391	17	19	2626	2704
3A-before	D2S1338	17	21	1928	2446

Sum of peak hts: 156984

Average (sum/46): 3413

3B-after	D3S1358	17	18	4663	4618
3B-after	vWA	17	18	3164	2193
3B-after	D16S539	11	12	2339	1514
3B-after	CSF1PO	10		3200	
3B-after	TPOX	8		1772	
3B-after	Yindel	2		6048	
3B-after	AMEL	X	Y	7313	5557
3B-after	D8S1179	10	14	6316	4873
3B-after	D21S11	30	32.2	2280	1990
3B-after	D18S51	16	18	2384	1657
3B-after	DYS391	10		2355	
3B-after	D2S441	11.3	14	5072	5089
3B-after	D19S433	13	15	4147	4542
3B-after	TH01	7	9	3719	3759
3B-after	FGA	18	25	3245	2855
3B-after	D22S1045	15	17	4357	3697
3B-after	D5S818	9	12	3712	3276
3B-after	D13S317	9	11	2993	3795
3B-after	D7S820	10	12	2268	1875
3B-after	SE33	21.2	27.2	1556	1645
3B-after	D10S1248	13		8616	
3B-after	D1S1656	12	17.3	3972	3704
3B-after	D12S391	17	19	2097	2178
3B-after	D2S1338	17	21	3071	3236

Sum of peak hts: 152712

Average (sum/46): 3320

Average peak height original amp (3A-before) 3413

Average peak height after GenTegra (3B-after) 3320

30% range from original amp 2390 to 4436  
 reamp after GenTegra within 30% yes

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**Biological Screening Lab Cleaning Log**

<b>Date /Initial</b>	<b>Task Completed</b>	<b>Comments</b>
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
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**DNA Casework Extraction Lab Cleaning Log**

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Date /Initial	Task Completed	Comments
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
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	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Biosafety hoods bleached (~every three months) <input type="checkbox"/> Common areas and equipment bleached (~monthly)	



## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

## PCR Lab Cleaning Log

Date /Initial	Task Completed	Comments
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	
	<input type="checkbox"/> Floors cleaned (~every three months) <input type="checkbox"/> Common areas bleached (every three months)	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**BIOLOGICAL SCREENING LAB INCUBATOR****Make/Model** \_\_\_\_\_**S/N** \_\_\_\_\_**Acceptable Temperature Range is 27°C to 47°C**

<b>Scientist</b>	<b>Date, Time &amp; Temperature at start of incubation</b>	<b>Date, Time &amp; Temperature at end of incubation</b>

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**CASEWORK EXTRACTION LAB INCUBATOR**

Model \_\_\_\_\_

S/N \_\_\_\_\_

**Acceptable Temperature Range is 50°C to 60°C**

<b>Scientist</b>	<b>Date, Time &amp; Temperature at start of incubation</b>	<b>Date, Time &amp; Temperature at end of incubation</b>



## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**THERMOMIXER TEMPERATURE LOG**

Model \_\_\_\_\_

S/N \_\_\_\_\_

Appropriate temperature set points are noted on instrument

Bi-yearly performance checks are documented at the bottom of page

Scientist	Date, Time & Set point at start of incubation	Date & Time at end of incubation

**Bi-annual performance checks**

Date	Analyst	56 C Set point	70 C Set point	Pass/Fail

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**pH Meter / Electrode Calibration Log**

- Prepare the balance for calibration as described in the electrode User Manual
- Record the following measurements

Date Calibrated	pH of Calibration buffer	Calibration buffer lot #	Calibration buffer expiration date	Successful calibration denoted by Scientist initials

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**Analytical Balance Performance Check**

- Check that balance is level and adjust if necessary
  - Weight set should be equilibrated to room temperature prior to use
  - Airflow in the room should be minimized
  - Weights should be taken while the scientist is seated, without applying pressure to the counter
  - The same door of the balance should be used throughout a weighing session
  - Weights should be handled with the supplied tweezers and always placed in the center of the weigh pan
  - The first weight should be placed and removed 3-5 times before recording the first measurement. This allows for the electronics of the balance to “warm up”
  - Ensure that the measurement has stabilized prior to recording
  - Ensure that the display is zeroed (while the door is closed) in between measurements
  - If the balance check fails at any weight, repeat the entire test. If it fails a second time, notify the DNA Technical Manager, who will determine an appropriate course of action
- 
- Record the following measurements

Weight Measured	Balance Readout (X.XXXX)	Acceptable Range	Result (pass/fail)
1g		0.9990-1.0010	
2g		1.9990-2.0010	
5g		4.9990-5.0010	
10g		9.9990-10.0010	

Troemner weight set (S/N 4000013561)

Date of last cal\_\_\_\_\_

Mettler Toledo XS204 Balance (S/N B208726643) Date of last cal\_\_\_\_\_

\_\_\_\_\_  
Scientist Signature\_\_\_\_\_  
Date Check Performed

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**EZ1 Advanced-XL-\_\_\_ Maintenance Log for Calendar Year \_\_\_\_\_****Alaska State Tag # \_\_\_\_\_****S/N: \_\_\_\_\_****Daily in use = wipe down, clean piercing units, check O-rings, and 30 minute UV**

Date in use	Task Completed (scientist initial in box)	Comments
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	
	<input type="checkbox"/> EZ1 maintenance/performance check completed <input type="checkbox"/> daily in use <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> preventative maintenance <input type="checkbox"/> service call	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**EZ1 ADVANCED-XL-\_\_\_ PIPETTING ACCURACY TEST****Alaska State Tag #\_\_\_\_\_ S/N \_\_\_\_\_**

**The following performance checks are to be performed approximately every 6 months. Upon completion of the tests, record the appropriate information for the laboratory balance and thermometer used in the spaces provided.**

1. The Pipetting Accuracy Test is performed using Qiagen Supplementary Protocol MA67.
2. Read the instructions completely prior to beginning the test. Perform both the 100 $\mu$ L and 500 $\mu$ L tests.
3. Record the weights in the tables below and calculate the weight differences.
4. If the robot does not pass one of these tests, repeat the test.
5. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

**Pipetting 100 $\mu$ L of water (acceptable range 92-108 $\mu$ L)**

<b>Tube</b>	<b>Weight before Run (g)</b>	<b>Weight after Run (g)</b>	<b>Difference (g)</b>	<b>Pipetted volume (<math>\mu</math>L)</b>	<b>Pass/Fail</b>
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

**Date:** \_\_\_\_\_ **Scientist:** \_\_\_\_\_

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**EZ1 ADVANCED-XL-\_\_\_ PIPETTING ACCURACY TEST****Pipetting 500 $\mu$ L of water (acceptable range 460-540 $\mu$ L)**

Tube	Weight before Run (g)	Weight after Run (g)	Difference (g)	Pipetted volume ( $\mu$ L)	Pass/Fail
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

Date: \_\_\_\_\_ Scientist: \_\_\_\_\_

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**EZ1 ADVANCED-XL-\_\_\_ LEAKAGE TEST**

1. The Leakage Test is performed using Qiagen Supplementary Protocol MA67.
2. Read the instructions completely prior to beginning the test.
3. Record the results in the space provided below.
4. There must be no dripping from the tips during the test.
5. If the robot does not pass this test, repeat the test.
6. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

<b>Tube</b>	<b>Tips dripped during run</b>	<b>Pass/Fail</b>
<b>1</b>		
<b>2</b>		
<b>3</b>		
<b>4</b>		
<b>5</b>		
<b>6</b>		
<b>7</b>		
<b>8</b>		
<b>9</b>		
<b>10</b>		
<b>11</b>		
<b>12</b>		
<b>13</b>		
<b>14</b>		

Date: \_\_\_\_\_ Scientist: \_\_\_\_\_

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**EZ1 ADVANCED-XL-\_\_\_\_ TEMPERATURE ACCURACY TEST**

1. The Temperature Accuracy Test is performed using Qiagen Supplementary Protocol MA68.
2. Read the instructions completely prior to beginning the test; be sure to wait the entire 20 minutes as described in Step 7 of the protocol.
3. Record the results in the space provided below.
4. If the measured temperature is within +/- 3°C, then the accuracy is within the defined specifications.
5. If the robot does not pass this test, repeat the test.
6. If the robot fails the test a second time, consult the Technical Manager to determine the appropriate course of action.

**Measured Temperature****Test Results  
(Pass/Fail)**

60°C

\_\_\_\_\_

\_\_\_\_\_

**Equipment used****Laboratory Balance**

Make/Model: \_\_\_\_\_

Serial Number: \_\_\_\_\_

Last Calibration Date: \_\_\_\_\_

**Thermometer**

Make/Model: \_\_\_\_\_

Serial Number: \_\_\_\_\_

Last Calibration Date: \_\_\_\_\_

**Scientist:** \_\_\_\_\_**Date:** \_\_\_\_\_

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**7500-\_\_ Real-Time PCR Maintenance Log for Calendar Year \_\_\_\_\_**  
**Alaska State Tag # \_\_\_\_\_**  
**S/N: \_\_\_\_\_**

Week of	Task Completed (scientist initial in box)	Comments
	<input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 daily in use maintenance completed <input type="checkbox"/> restart <input type="checkbox"/> wipe clean <input type="checkbox"/> 7500 monthly maintenance completed <input type="checkbox"/> background <input type="checkbox"/> disk defrag/cleanup <input type="checkbox"/> Lamp: Good / Failed / Change soon <input type="checkbox"/> Usage (hours): _____ <input type="checkbox"/> 7500 semi-annual maintenance completed <input type="checkbox"/> ROI <input type="checkbox"/> background <input type="checkbox"/> optical <input type="checkbox"/> RNase P <input type="checkbox"/> FAM <input type="checkbox"/> VIC <input type="checkbox"/> ABY <input type="checkbox"/> JUN <input type="checkbox"/> Mustang Purple <input type="checkbox"/> other <input type="checkbox"/> service / PM date: _____ <input type="checkbox"/> PC pass date: _____	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 daily in use maintenance completed <input type="checkbox"/> restart <input type="checkbox"/> wipe clean <input type="checkbox"/> 7500 monthly maintenance completed <input type="checkbox"/> background <input type="checkbox"/> disk defrag/cleanup <input type="checkbox"/> Lamp: Good / Failed / Change soon <input type="checkbox"/> Usage (hours): _____ <input type="checkbox"/> 7500 semi-annual maintenance completed <input type="checkbox"/> ROI <input type="checkbox"/> background <input type="checkbox"/> optical <input type="checkbox"/> RNase P <input type="checkbox"/> FAM <input type="checkbox"/> VIC <input type="checkbox"/> ABY <input type="checkbox"/> JUN <input type="checkbox"/> Mustang Purple <input type="checkbox"/> other <input type="checkbox"/> service / PM date: _____ <input type="checkbox"/> PC pass date: _____	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> 7500 daily in use maintenance completed <input type="checkbox"/> restart <input type="checkbox"/> wipe clean <input type="checkbox"/> 7500 monthly maintenance completed <input type="checkbox"/> background <input type="checkbox"/> disk defrag/cleanup <input type="checkbox"/> Lamp: Good / Failed / Change soon <input type="checkbox"/> Usage (hours): _____ <input type="checkbox"/> 7500 semi-annual maintenance completed <input type="checkbox"/> ROI <input type="checkbox"/> background <input type="checkbox"/> optical <input type="checkbox"/> RNase P <input type="checkbox"/> FAM <input type="checkbox"/> VIC <input type="checkbox"/> ABY <input type="checkbox"/> JUN <input type="checkbox"/> Mustang Purple <input type="checkbox"/> other <input type="checkbox"/> service / PM date: _____ <input type="checkbox"/> PC pass date: _____	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

7500-\_\_\_ Standard Curve Y-Intercepts – Calendar Year \_\_\_\_\_

Alaska State Tag # \_\_\_\_\_

S/N: \_\_\_\_\_

Date	Analyst	Kit Lot #	Ver / CW / DB	Y-intercept			Within +/- 1 of verified?
				T-Male	T-Large	T- Small	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**ProFlex Thermal Cycler-\_\_ Maintenance Log for Calendar Year \_\_\_\_\_****Alaska State Tag # \_\_\_\_\_****Base S/N \_\_\_\_\_****Block S/N \_\_\_\_\_**

**Monthly = cleaning wells, cleaning cover, and Self Verification Test**  
**Performance Check following PM or service = Self Verification Test and**  
**amplification of positive and negative amp control**

Name	Date	Maintenance Performed
		<input type="checkbox"/> Maintenance performed and passed <input type="checkbox"/> monthly <input type="checkbox"/> PC following PM/service <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other
		<input type="checkbox"/> Maintenance/performance check passed <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> service call/ other

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**3500xl-\_\_ Maintenance Log for Calendar Year \_\_\_\_\_****Alaska State Tag # \_\_\_\_\_*****Capillary changes documented on Capillary Change Form***

Date	Task Completed (scientist initial in box)	Lot # / Expiration / Comments
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> Daily in use maintenance – wipe and restart Reagent changed: <input type="checkbox"/> ABC <input type="checkbox"/> CBC <input type="checkbox"/> POP-4 Monthly: <input type="checkbox"/> defrag <input type="checkbox"/> water wash <input type="checkbox"/> trap flush <input type="checkbox"/> quarterly performance check – doc'n in binder <input type="checkbox"/> service call	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

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**3500xl-\_\_ Capillary Change / post-annual PM in Calendar Year\_\_\_\_\_****Alaska State Tag # \_\_\_\_\_**

***This form must be completed and submitted with all accompanying documentation for TM (or designee) approval before the instrument can be put online following a capillary change or completion of annual preventative maintenance or service which affects instrument optics.***

**Scientist: \_\_\_\_\_****Date of capillary change: \_\_\_\_\_****New Capillary Lot Number: \_\_\_\_\_****Dye Set J6 Lot # \_\_\_\_\_ Exp: \_\_\_\_\_****Dye Set Promega G5 Lot #\_\_\_\_\_ Exp. \_\_\_\_\_****Scientist date and initial for each task:**\_\_\_\_\_ **Water wash and water trap flush**\_\_\_\_\_ **Spectral for Dye Set J6 passed**\_\_\_\_\_ **Spectral for dye set J6-OSR passed; report attached**\_\_\_\_\_ **Spectral for Dye set Promega G5 passed; report attached**\_\_\_\_\_ **Performance check passed; report attached (confirm on printout that performance check included a spatial and a J6 spectral)**

**Documentation is complete and correct; this instrument may be put back online for use in casework and database analysis as of \_\_\_\_\_ .**

**Authorized by: \_\_\_\_\_**

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**QIACube-\_\_\_\_\_ Maintenance Log for Calendar Year \_\_\_\_\_**  
**Alaska State Tag # \_\_\_\_\_**  
**S/N: \_\_\_\_\_**

Date	Task Completed (scientist initial in box)	Comments
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	
	<input type="checkbox"/> instrument in use <input type="checkbox"/> Maintenance completed <input type="checkbox"/> regular <input type="checkbox"/> monthly <input type="checkbox"/> bi-annual <input type="checkbox"/> performance check pass – documentation in binder <input type="checkbox"/> service call	

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

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**POST-PCR PLATE HEAT BLOCK TEMPERATURE LOG**

Model: \_\_\_\_\_

S/N: \_\_\_\_\_

Temperature set point: \_\_\_\_\_

**Acceptable Thermometer temperature range is 95 - 99°C**

Analyst	Date	Time	Side of heat block	Thermometer temperature	Pass / Fail

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

Version:3.0

**Appendix A: Revision History**

<b>FBGLM 3.0 Page</b>	<b>FBGLM 2.0 Page</b>	<b>Location</b>	<b>Revision made</b>
<b>NA</b>	<b>NA</b>	<b>throughout</b>	Grammar, spelling corrections, page numbers updated
<b>NA</b>	<b>NA</b>	<b>throughout</b>	<b>Updated</b> all procedures, logs, and references to 9700 thermal cyclers with procedures, logs, and references to ProFlex thermal cyclers.
<b>NA</b>	<b>NA</b>	<b>throughout</b>	<b>Replaced</b> FBCP with FBPM
<b>7-13</b>	<b>7-13</b>	<b>Section 1.4</b>	<b>Added first bullet point:</b> "Verification must be performed using the most stringent conditions routinely encountered in casework, including Gentegra dry-down and re-hydration in minimum amplification volume where applicable." <b>Added</b> to relevant verification procedures.
<b>8</b>	<b>8</b>	<b>Section 1.4</b>	<b>Added</b> details on expiration dates for GenTegra-DNA
<b>9</b>	<b>9</b>	<b>Section 1.4</b>	<b>Clarified</b> storage conditions for GlobalFiler reagents
<b>42</b>	<b>42</b>	<b>GenTegra verification form</b>	In step 5, <b>removed</b> reference to cover page.
<b>66</b>	<b>66</b>	<b>Post-PCR Heat Block log</b>	<b>Modified</b> to make suitable for use with either model of heat block

## Forensic Biology General Lab Maintenance Manual

Effective:3/14/2022

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**Appendix B: Program for the calibration of equipment**

<b>Equipment requiring calibration</b>	<b>Specifications for the calibration laboratory</b>	<b>Specified requirements for the calibration</b>	<b>Interval of calibration</b>
Analytical Balance	ISO 17025 accreditation with scope covering this equipment type	2 mg to 50 g	Annual
Weight set for analytical balance	ISO 17025 accreditation with scope covering this equipment type	Range specified by manufacturer	Annual
Pipettes	ISO 17025 accreditation with scope covering this equipment type	Pipettes are calibrated to the range specified by the manufacturer and labeled on each pipette	Annual
Temperature probes (post-PCR)	ISO 17025 accreditation with scope covering this equipment type	Range specified by manufacturer	Annual
Thermometers and pre-PCR room temperature probes	NA – thermometers outside manufacturer designated calibration interval are discarded	Thermometers must be chosen such that their calibration range encompasses the range specified on its corresponding instrument temperature log	Thermo-mixer / EZ1 temperature probes purchased as needed; calibration interval based on certificate provided with purchased thermometer
pH meter and electrode	Performed in house	Range specified by manufacturer	Calibration performed before each use
Applied Biosystems 7500 Real-Time PCR System	Manufacturer of the instrument	Conditions specified by manufacturer	Calibrations performed monthly and/or semi-annually, based on calibration type. If in-house calibration does not pass, vendor is contacted to make repairs or adjustments as needed
Applied Biosystems 3500xL Genetic Analyzers	Manufacturer of the instrument	Conditions specified by manufacturer	Spectral and spatial calibrations are performed when array is replaced (or following PM if optics affected. If in-house calibration does not pass, vendor is contacted to make repairs or adjustments as needed

## Forensic Biology General Lab Maintenance Manual

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**Appendix C: Performance Check program for critical instruments**

***In addition to new critical instruments requiring performance checks, and performance checks required following service or preventive maintenance performed by an outside vendor, critical instruments require performance checks on the schedule described in the table below.***

Instrument	Frequency of performance checks	Performed by	Contents of performance check
Handheld mechanical pipettes	<a href="#">Annual</a>	Outside vendor	Vendor follows their own protocols for evaluating at the high and low settings of each pipette's range
All other thermometers	<a href="#">Annual</a>	Outside vendor	NIST-traceable thermometers are purchased annually; certificate of calibration included with new pipette serves as performance check
Thermomixers	<a href="#">Bi-annual</a>	Lab technician or analyst	NIST-traceable thermometer is used to monitor temperature settings
EZ1 BioRobots	<a href="#">Bi-annual</a>	Lab technician or analyst	Pipette accuracy, Leakage, and Temperature accuracy
QIAcube	<a href="#">Monthly and Bi-annual</a>	Lab technician or analyst	Monthly tightness test; bi-annual extraction of a known sample (non-sperm + sperm)
7500 RT-PCR Instruments	<a href="#">Bi-annual</a>	Outside vendor and Lab technician or analyst	Thermal cycler temperature verification is performed and evaluated as part of annual PM by vendor; standard curve and NTC run following service
ProFlex Thermal Cyclers	<a href="#">Annual</a>	Outside vendor	Temperature Calibration Verification, Temperature Non-Uniformity Test and the Hardware Diagnostics/System Performance Tests
Post-PCR plate prep heat block	<a href="#">Before each use</a>	Lab technician or analyst	Analyst checks set temperature against a NIST-traceable thermometer
3500 Genetic analyzers	<a href="#">Every three months and after each capillary change</a>	Lab technician or analyst	HID Install standard protocol